Effects of frequency of supplementation on dry matter intake and net portal and hepatic flux of nutrients in mature ewes that consume low-quality forage

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Effects of Frequency of Supplementation on Dry Matter Intake and Net Portal and Hepatic Flux of Nutrients in Mature Ewes That Consume Low-Quality Forage[1,2]

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ABSTRACT: Our objective was to determine the effects of frequency of soybean meal (SBM) supplementation on forage intake and net portal-drained viscera (PDV) and hepatic flux of nutrients in ewes that consume low-quality forage. Six Polled Dorset ewes (BW ± SD = 82 ± 9 kg) fitted with hepatic venous, hepatic portal, abdominal aortic, and mesenteric venous catheters were used in a replicated 3 × 3 Latin square design. Ewes consumed bromegrass hay (7.5% CP; DM basis). Treatments were no supplement (control), SBM fed once every 24 h, or SBM fed once every 72 h. In the SBM treatments, SBM was fed to provide 80 g/d of CP. Blood flow and net flux measurements were made on the 3rd d of each period so that ewes supplemented every 72 h were sampled the day of, the day after, and 2 d after supplementation. Arterial concentrations of α-amino N (AAN) and ammonia N were lower ($P < .01$) when SBM was fed, whereas arterial concentrations of urea N and oxygen were higher ($P < .01$). Feeding SBM increased net PDV release of AAN and ammonia N, net PDV removal of urea N, and oxygen consumption. A SBM × sampling day interaction ($P < .05$) occurred and resulted in greater net PDV absorption of AAN and ammonia N on the day after SBM supplementation when ewes were fed SBM on a 72-h interval. Net hepatic removal of AAN, ammonia N, and oxygen, and net hepatic release of urea N were greater ($P < .01$) with feeding SBM. The results indicate that the interval of SBM supplementation may affect the pattern of absorption without affecting the net absorption of nutrients.

Key Words: Ewes, Forage, Protein Supplements, Nutrient Flux

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Introduction

The low CP content of dormant forages results in decreased BW in ruminants that consume winter range. Therefore, feeding high-CP supplements improved ADG (McCollum and Galyean, 1985; Delcurto et al., 1990a; Beaty et al., 1994) and increased DMI (Lee et al., 1985) and DM digestion. A common practice among range livestock producers is to feed supplemental protein at 48- or 72-h intervals to increase grazing time and reduce labor costs when cows graze low-quality winter forage. Several studies have shown no detrimental effect on animal performance when protein supplements were fed at 48-h (Hunt et al., 1989), 72-h (McIlvain and Shoop, 1963), or 96-h (Coleman and Wyatt, 1982) intervals. Nolan and Leng (1972) suggested that recycling of absorbed ammonia to the rumen may support fermentation between times of supplementation. However, the physiological mechanisms associated with maintained performance with infrequent protein supplementation have not been elucidated. Therefore, this experiment was conducted to determine the effects of frequency of protein supplementation on forage intake and net portal and hepatic flux of nutrients in ewes that consume low-quality hay.

Materials and Methods

Six mature polled Dorset ewes (BW ± SD = 82 ± 9 kg) from the U.S. Meat Animal Research Center flock were surgically fitted with chronic indwelling catheters in a hepatic vein, the portal vein, a mesenteric vein, and the abdominal aorta (Ferrell et

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[2]Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the USDA or an endorsement over products not mentioned.

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al., 1991). Ewes were housed in individual pens (1.17 m × 2.34 m) at 20°C with a light:dark cycle of 14:10 h. Protocols were in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Consortium, 1988). Protocols were approved by the Institutional Animal Care and Use Committee of the Center.

Ewes had ad libitum access to bromegrass hay (7.5% CP, 72.6% NDF; DM basis). The bromegrass hay was harvested in September (a first cutting hay), baled, and stored in the field for 8 mo. Ewes were fed daily at 0730, and feed refusal from the previous day was determined before feeding. Ewes had ad libitum access to water. Five grams of salt and 1 g of a mineral mix (contained 14% Ca, 12% Zn, 8% Mn, 10% Fe, .2% I, and .1% Co) were fed daily. Treatments were control (bromegrass hay alone), soybean meal (**SBM**) fed once every 24 h (**ED**), or SBM fed once every 72 h (**ETD**). Soybean meal was fed to provide an additional 80 g/d of CP and provided an additional .52 Mcal/d of ME above that provided by bromegrass hay. The amount of SBM fed was based on estimated intake and protein requirements for mature ewes (NRC, 1985). The SBM was fed in separate feeders to promote complete and rapid consumption. Feed samples were analyzed for DM and N (AOAC, 1990) and also for NDF (Goering and Van Soest, 1970).

The ewes were allotted to a replicated 3 × 3 Latin square design with 21-d periods (i.e., 12 d for adaptation and 9 d for sampling), and the experiment was balanced for residual effects (Cochran and Cox, 1957). The DMI was measured from d 13 to 21 of each period. Simultaneous arterial, portal, and hepatic blood samples were taken five times at 40-min intervals beginning at 0900 on d 13, 14, 18, 19, 20, and 21. Replicate 1 was bled on d 13, 18, and 20, and Replicate 2 was bled on d 14, 19, and 21. This design allowed us to sample ewes supplemented once every 72 h on d 0, 1, and 2 after supplementation (i.e., ewes supplemented once every 72 h were sampled at 1.5, 25.5, and 49.5 h of supplementation). During blood sampling, ewes were placed in portable crates (.04 m wide × 1.2 m long × .117 m high). Portal and hepatic blood and plasma flows were measured by the downstream dilution of a primed (15 mL) continuous infusion (.80 mL/min) of para-aminohippurate (**PAH**; 5% wt/vol, pH 7.4) infused through a sterile .45-μm filter. Para-aminohippurate infusion began 60 min before blood sampling. Ten milliliters each of arterial, portal venous, and hepatic venous blood was collected into heparinized syringes containing 12 mg NaF and immediately placed on ice for transporting to the laboratory. An additional 2 mL of blood was anaerobically drawn into heparinized syringes and analyzed immediately for hemoglobin, percentage of oxygen saturation of hemoglobin (Hemoximeter, Model OSM-2, Radiometer America, Westlake, OH), L-lactate (Model 27 YSI, Yellow Springs Instrument, Yellow Springs, OH), and packed cell volume.

In the laboratory, 2.5 mL of blood was frozen (−20°C) immediately. On the day of sampling, an additional 1 mL of blood was mixed with 2 mL of H_2O that contained heparin (20 U/mL) for analysis of PAH (Harvey and Brothers, 1962), α-amino N (**AAN**; Palmer and Peters, 1969), urea N (Marsh et al., 1965), and ammonia N (Huntington, 1982) using a Technicon Autoanalyzer System (Technicon Autoanalyzer Systems, Tarrytown, NY). The remaining blood (6.5 mL) was centrifuged (15,000 × *g*, 15 min at 4°C), and plasma was harvested and then frozen (−20°C). Plasma samples were assayed for glucose (Gochmann and Schmitz, 1972) and PAH as described previously.

Oxygen concentrations were calculated (Burrin et al., 1989). Plasma and whole blood flow rates through the portal-drained viscera (**PDV**) and liver and net flux of nutrients across the PDV, hepatic, and splanchnic vascular beds also were calculated (Krehbiel et al., 1992). A positive number indicates a net release, whereas a negative number indicates a net uptake.

Arithmetic means for blood flow and nutrient concentration were generated for each ewe within each sampling day by averaging the values obtained from each blood sample. The experiment was analyzed as a replicated Latin square using the GLM procedures of SAS (1990). The model included square, ewe within square, period, and treatment. Two contrast comparisons were constructed to evaluate supplementation effects: 1) no SBM vs SBM and 2) SBM supplementation ED (24 h) vs ETD (72 h). Sampling day was analyzed using split-plot analysis. Sums of squares owing to ewe × period × treatment were used as the whole-plot error term to test for significance of SBM effects. The residual sums of squares were used as the subplot error term to test for significance of sampling day and the SBM × sampling day interaction. Results were considered significant at the $P < .05$ level.

Results

One ewe fed SBM ETD during Period 2 stopped eating, which resulted in a missing observation for that treatment. Dry matter intake of bromegrass hay tended ($P = .07$) to increase with feeding SBM (Table 1). Across sampling days, ewes that consumed bromegrass or bromegrass supplemented with SBM ED, or ETD consumed 613, 689, and 641 g/d of bromegrass (DM basis), respectively. Feeding SBM ED or ETD increased ($P < .01$) intake of N and ME. Ewes fed bromegrass alone or with SBM ED consumed an average of 47 and 133 g/d of CP and 1.50 and 2.19 Mcal/d of ME, respectively. Ewes fed bromegrass supplemented with SBM ETD consumed 281 g of CP and 3.0 Mcal of ME on the day of supplementation and an average of 51 g of CP and 1.61 Mcal of ME during

Table 1. Effects of soybean meal (SBM) and frequency of its supplementation on DM, N, and ME intake by ewes that consumed bromegrass hay

Item	No supplement			SBM, 24 h[a]			SBM, 72 h[b]				Contrasts,[c] *P*-value	
	D1[d]	D2[d]	D3[d]	D1	D2	D3	D1	D2	D3	SEM[e]	NS vs S	24 vs 72 h
DMI, g/d												
Bromegrass hay	614	616	609	692	698	679	597	655	672	40	.07	.15
SBM	0	0	0	162	162	162	486	0	0	—	—	—
Total[f]	614	616	609	854	860	841	1083	655	672	31	<.01	.07
N intake, g/d[f]	7.4	7.7	7.5	21.2	21.4	20.9	44.9	8.1	8.2	.4	<.01	.06
ME intake, Mcal/d[fg]	1.49	1.50	1.48	2.20	2.21	2.16	3.00	1.59	1.63	.10	<.01	.15

[a]SBM was fed once every 24 h.
[b]SBM was fed once every 72 h.
[c]NS vs S = no supplement vs SBM supplementation; 24 vs 72 h = feeding SBM once every 24 h or every 72 h.
[d]D1, D2, and D3 represent sampling d 1, 2, and 3. All ewes were sampled 3 d in each period so that ewes fed SBM once every 72 h were sampled the day of, the day after, and the 2nd d after supplementation.
[e]Pooled standard error of the least squares means; n = 6, except for n = 5 for the group fed SBM every 72 h.
[f]SBM × sampling day interaction ($P < .05$).
[g]Based on tabular values (NRC, 1985).

the following 2 d. By design, a SBM × sampling day interaction ($P < .01$) was observed for total DM, N, and ME intakes. These interactions can be explained by observed increases in total DM, N, and ME intakes the day of vs the day after or 2 d after supplementation when ewes were fed SBM ETD.

Arterial concentration of AAN was lower ($P < .01$) with feeding SBM (Table 2). Portal-arterial (**PA**) concentration difference of AAN was positive ($P < .01$) across all treatments, indicating net release from the PDV. Portal-arterial concentration difference of AAN was greater ($P < .01$) when SBM was fed. A SBM × sampling day interaction ($P < .05$) occurred for PA concentration difference of AAN. This resulted in greater release of AAN by the PDV on the day after supplementation of SBM on a 72-h interval. Hepatic-arterial (**HA**) concentration difference of AAN was less negative ($P < .05$) when SBM was fed. Across treatments, hepatic-portal (**HP**) concentration difference of AAN was negative and different ($P < .01$) from zero and, in general, mirrored AAN PA concentration differences. Greater ($P < .01$) removal of AAN by the liver occurred when SBM was fed.

Regardless of feeding frequency, arterial concentration of ammonia N was reduced ($P < .01$) from .220 to .188 m*M* (i.e., 14%) when SBM was fed (Table 2). Portal-arterial concentration differences were positive, indicating net release of ammonia N by the PDV. Ammonia N PA concentration difference was greater ($P < .01$) when SBM was fed. Hepatic-arterial concentration difference of ammonia N did not differ ($P > .05$) among treatments, suggesting that ammonia N did not escape the liver as free ammonia. The negative HP concentration difference of ammonia N, similar in magnitude to the PA difference, indicated that all ammonia N absorbed across the PDV was removed by the liver.

Arterial concentration of urea N was doubled ($P < .01$) by feeding SBM compared with control (Table 2). In addition, arterial concentration of urea N tended ($P = .09$) to be greater with feeding SBM ETD than ED. Soybean meal × sampling day interactions ($P < .05$) occurred for arterial urea N and HA and HP concentration differences ($P < .05$). When ewes were fed bromegrass or bromegrass with SBM ED, arterial urea N and urea N HA and HP concentration differences were similar across the three sampling days. In contrast, when ewes were fed SBM ETD, arterial urea N and HA and HP concentration differences increased between sampling d 1 and 2. In general, greater urea N arterial concentration ($P < .01$) and PA ($P < .05$), HA ($P < .01$), and HP ($P < .01$) concentration differences were observed with feeding SBM.

Arterial concentration and PA, HA, and HP concentration differences of glucose were not affected ($P > .05$) by SBM supplementation (Table 2). Lactate arterial concentration also was not affected by SBM ($P > .05$) or sampling day ($P > .05$). Portal-arterial concentration difference of lactate was greater ($P < .05$) when SBM was fed, whereas HA ($P < .01$) and HP ($P = .01$) concentration differences of lactate were lower. In addition, HA concentration difference of lactate tended ($P = .07$) to increase with feeding SBM ETD vs ED. Arterial concentration and PA and HP concentration differences of lactate were not affected ($P > .05$) by day of sampling.

Arterial concentration and HA concentration difference of oxygen responded with a SBM × sampling day interaction ($P < .05$; Table 2) and resulted in an increase in arterial concentration and HA concentration difference of oxygen on sampling d 2 for ewes fed SBM. Oxygen arterial concentration and PA, HA, and HP concentration differences were greater ($P < .01$) when SBM was fed. No effects of day of sampling ($P > .05$) were observed for arterial concentration or PA or HA concentration differences of oxygen. Hepatic-portal concentration difference of oxygen tended ($P = .08$) to increase with feeding SBM ETD vs ED.

Portal and hepatic venous blood flows were not affected by the treatments (data not shown). Table 3 shows a SBM × sampling day interaction ($P < .05$) for net PDV and splanchnic release of AAN. Portal-drained viscera release of AAN was similar across sampling days when ewes were fed bromegrass or bromegrass plus SBM ED. However, when ewes were fed bromegrass plus SBM ETD, PDV flux of AAN increased between sampling d 1 and 2 and then decreased on d 3 to release rates similar to those of ewes fed SBM ED. Total splanchnic release of AAN was greater on d 2 of sampling for ewes fed SBM ETD. The net release of AAN on d 2 (5.6 mmol/h) in ewes fed SBM ED was not different from zero ($P > .05$). In the control ewes, total splanchnic removal of AAN decreased from sampling d 1 to 3. Net PDV release, hepatic uptake, and hepatic extraction of AAN were greater ($P < .01$) when SBM was fed. Net PDV, hepatic, and total splanchnic flux and hepatic extraction ratio were not affected ($P > .05$) by sampling day.

Flux data for ammonia N were similar to AAN data (Table 3). Net PDV release and net hepatic removal of ammonia N showed a SBM × sampling day interaction ($P < .05$) similar to that for AAN. Portal-drained viscera release and hepatic removal of ammonia N were similar across sampling days when ewes were fed bromegrass alone or with SBM ED. However, when ewes were fed SBM ETD, PDV release and hepatic uptake of ammonia N increased between sampling d 1 and 2 and then decreased. Net PDV

Table 2. Effects of soybean meal (SBM) and frequency of its supplementation on arterial concentrations and venous-arterial differences for metabolites in ewes that consumed bromegrass hay

	No supplement			SBM, 24 h[a]			SBM, 72 h[b]				Contrasts,[c] *P*-value	
Item	D1[d]	D2[d]	D3[d]	D1	D2	D3	D1	D2	D3	SEM[e]	NS vs S	24 vs 72 h
	mM											
α-Amino N												
Arterial	5.50	5.68	5.49	4.62	4.81	4.85	4.84	4.81	4.70	.14	<.01	.86
P-A[fg]	.10	.06	.10	.25	.28	.26	.16	.36	.23	.04	<.01	.71
H-A[gh]	–.05	–.02	–.01	–.01	.04	.01	–.04	.07	–.00	.02	.02	.87
H-P[f]	–.15	–.09	–.10	–.27	–.24	–.25	–.20	–.29	–.23	.04	<.01	.76
Ammonia N												
Arterial	.211	.232	.216	.172	.203	.185	.188	.187	.193	.012	<.01	.81
P-A	.060	.061	.063	.190	.189	.194	.133	.220	.156	.016	<.01	.13
H-A	–.005	–.005	–.004	–.004	–.003	–.003	–.006	–.003	–.006	.002	.86	.53
H-P	–.065	–.066	–.066	–.194	–.194	–.197	–.139	–.223	–.162	.015	<.01	.12
Urea N												
Arterial[g]	2.41	2.36	2.16	4.49	4.59	4.72	3.45	6.92	5.04	.35	<.01	.09
P-A	–.03	–.06	–.05	–.08	–.04	–.08	–.08	–.09	–.07	.02	.02	.31
H–A[g]	.08	.06	.05	.16	.22	.15	.10	.28	.15	.02	<.01	.95
H-P[g]	.11	.12	.09	.23	.26	.23	.18	.36	.22	.02	<.01	.54
Glucose												
Arterial	4.27	4.29	4.05	4.28	4.42	4.18	4.43	4.96	4.30	.26	.21	.23
P-A	.02	–.03	–.06	–.03	–.13	–.05	–.03	–.04	–.03	.06	.53	.48
H-A	.25	.21	.02	.16	.26	.21	.14	.25	.25	.05	.17	.99
H-P	.23	.24	.07	.20	.38	.26	.17	.29	.28	.09	.22	.66
Lactate												
Arterial	.59	.60	.54	.61	.66	.65	.72	.59	.55	.05	.18	.71
P-A	.05	.09	.08	.08	.10	.09	.09	.08	.08	.01	.03	.50
H-A	–.12	–.13	–.11	–.06	–.01	–.05	–.06	–.06	–.10	.02	<.01	.07
H-P	–.17	–.21	–.18	–.14	–.11	–.14	–.15	–.14	–.18	.02	.01	.26
Oxygen												
Arterial[g]	5.42	5.49	4.95	6.06	6.32	6.09	5.91	6.23	5.79	.21	<.01	.33
P-A	–.94	–1.03	–1.06	–1.27	–1.38	–1.42	–1.18	–1.35	–1.22	.09	<.01	.16
H-A[g]	–1.77	–1.91	–1.88	–2.26	–2.44	–2.32	–2.24	–2.54	–2.35	.12	<.01	.71
H-P	–.83	–.88	–.83	–.99	–1.06	–.90	–1.05	–1.20	–1.13	.09	<.01	.08

[a]SBM was fed once every 24 h.
[b]SBM was fed once every 72 h.
[c]NS vs S = no supplement vs SBM supplementation; 24 vs 72 h = feeding SBM once every 24 h or every 72 h.
[d]D1, D2, and D3 represent sampling d 1, 2, and 3. All ewes were sampled 3 d in each period so that ewes fed SBM once every 72 h were sampled the day of, the day after, and the 2nd d after supplementation.
[e]Pooled standard error of the least squares means; n = 6 except for n = 5 for the group fed SBM every 72 h.
[f]P-A = portal-arterial concentration difference; H-A = hepatic-arterial concentration difference; H-P = hepatic-portal concentration difference.
[g]SBM × sampling day interaction ($P < .05$).
[h]Sampling day effect ($P < .01$).

absorption, hepatic uptake, and liver extraction of ammonia N were greater ($P < .01$) when ewes were fed SBM. Total splanchnic flux of ammonia N was not affected ($P > .05$) by SBM supplementation. Net PDV, hepatic, and total splanchnic flux and hepatic extraction of ammonia N were not affected ($P > .05$) by frequency of SBM supplementation.

Net PDV removal of urea N was greater ($P < .05$) when ewes were fed SBM (Table 3). In addition, there was a tendency ($P = .10$) for urea N removal by the PDV to be greater when SBM was fed ETD (−17.5 mmol/h) than ED (−12.2 mmol/h). Net hepatic and total splanchnic flux of urea N showed a SBM × day of sampling interaction ($P < .05$). The pattern of liver urea N release was a mirror image of the pattern of PDV absorption and hepatic removal of AAN and ammonia N. Net hepatic and total splanchnic flux of urea N were greater ($P < .01$) when SBM was fed. However, net hepatic and total splanchnic flux of urea N were not influenced ($P > .05$) by frequency of SBM supplementation. Hepatic extraction ratio of urea N was not affected ($P > .05$) by SBM supplementation or frequency of SBM supplementation.

Net PDV flux, hepatic flux, and hepatic extraction ratio of glucose were not affected ($P > .05$) by SBM or frequency of its supplementation (Table 3). Total splanchnic flux of glucose tended ($P = .09$) to be greater when ewes were fed SBM. Net PDV lactate release was greater ($P = .05$) in ewes fed SBM. When ewes were fed SBM ED or ETD, net hepatic flux, total splanchnic flux, and hepatic extraction of lactate were decreased ($P < .05$). Feeding ewes SBM ED or ETD

Table 3. Effects of soybean meal (SBM) and frequency of its supplementation on net portal, hepatic, and total splanchnic flux of metabolites in ewes that consumed bromegrass hay

	No supplement			SBM, 24 h[a]			SBM, 72 h[b]				Contrasts,[c] *P*-value	
Item	D1[d]	D2[d]	D3[d]	D1	D2	D3	D1	D2	D3	SEM[e]	NS vs S	24 vs 72 h
	mmol/h											
α-Amino N												
PDV[fg]	17.7	8.4	16.4	47.9	49.9	45.0	31.5	81.0	43.1	9.4	<.01	.61
Hepatic	−26.9	−13.7	−17.6	−51.1	−44.3	−46.4	−41.8	−64.9	−44.9	9.3	<.01	.69
TS[fg]	−9.2	−5.3	−1.2	−3.2	5.6	−1.4	−10.4	16.1	−1.7	4.6	.07	.81
HER[f]	−3.7	−1.3	−1.7	−5.7	−3.8	−4.8	−4.9	−4.5	−4.8	1.0	<.01	.97
Ammonia N												
PDV[g]	11.2	10.6	11.7	37.1	38.0	38.8	26.8	51.5	30.3	4.3	<.01	.64
Hepatic[g]	−12.2	−11.8	−12.7	−38.2	−38.7	−39.2	−28.2	−51.9	−31.6	4.1	<.01	.69
TS	−1.1	−1.2	−.9	−1.0	−.7	−.5	−1.4	−.4	−1.4	.5	.64	.40
HER	−26.6	−23.7	−25.6	−55.9	−51.7	−52.3	−48.3	−56.0	−51.0	2.2	<.01	.44
Urea N												
PDV	−6.1	−12.0	−10.0	−14.3	−8.0	−14.3	−16.5	−20.7	−15.2	3.6	.04	.10
Hepatic[g]	23.7	25.5	20.0	52.3	63.5	49.0	39.0	91.5	48.3	6.1	<.01	.39
TS[g]	17.7	13.4	10.0	38.0	55.5	34.7	22.5	70.8	33.0	7.8	<.01	.89
HER	8.9	8.5	7.0	8.9	10.7	8.5	8.1	8.7	7.8	1.0	.34	.19
Glucose												
PDV	2.5	−5.5	−24.6	−7.1	−14.5	−7.3	−2.0	−6.6	−4.7	13.6	.82	.66
Hepatic	34.0	42.0	26.1	37.7	57.3	39.7	26.5	55.9	46.4	17.9	.43	.90
TS	36.5	36.5	1.5	30.5	42.8	32.4	24.5	49.3	41.7	10.0	.09	.71
HER	11.6	10.6	2.0	8.3	14.7	11.3	9.3	10.8	12.3	.3	.18	.82
Lactate												
PDV	10.5	16.6	14.9	16.8	19.2	16.7	17.5	18.6	16.1	2.5	.05	.93
Hepatic	−34.8	−45.7	−37.1	−31.2	−19.7	−26.6	−27.5	−32.1	−37.7	6.0	.02	.21
TS	−24.4	−29.1	−22.2	−14.4	−.5	−9.9	−10.0	−13.5	−21.6	4.5	<.01	.09
HER	−52.3	−60.0	−53.7	−34.1	−20.6	−29.0	−25.7	−30.6	−47.9	6.8	<.01	.25
Oxygen												
PDV	−168	−179	−201	−247	−244	−248	−231	−303	−234	19	<.01	.55
Hepatic[g]	−219	−253	−231	−300	−354	−271	−278	−343	−286	27	<.01	.79
TS[g]	−387	−432	−433	−547	−598	−519	−509	−646	−520	31	<.01	.90
HER	−52.0	−55.2	−59.6	−58.1	−60.2	−57.7	−60.1	−65.6	−65.2	.4	.05	.16

[a]SBM was fed once every 24 h.
[b]SBM was fed once every 72 h.
[c]NS vs S = no supplement vs SBM supplementation; 24 vs 72 h = feeding SBM once every 24 h or every 72 h.
[d]D1, D2, and D3 represent sampling d 1, 2, and 3. All ewes were sampled 3 d in each period so that ewes fed SBM once every 72 h were sampled the day of, the day after, and 2nd d after supplementation.
[e]Pooled standard error of the least squares means; n = 6 except for n = 5 for the group fed SBM every 72 h.
[f]PDV = portal-drained viscera; TS = total splanchnic; HER = hepatic extraction ratio.
[g]SBM × sampling day interaction ($P < .05$).

did not affect ($P > .05$) net PDV flux, net hepatic flux, or hepatic extraction ratio of lactate. Total splanchnic flux of lactate tended ($P = .09$) to be greater when SBM was fed ETD (−15.0 mmol/h) than ED (−8.2 mmol/h).

Net PDV, hepatic, and total splanchnic consumption ($P < .01$) and hepatic extraction ratio ($P = .05$) of oxygen were greater when ewes were fed SBM (Table 3). Net hepatic and total splanchnic consumption of oxygen showed a SBM × sampling day interaction ($P < .05$) and resulted in greater oxygen removal by the liver and total splanchnic tissues on d 2 of sampling compared with d 1 or 3 when SBM was fed. Frequency of supplementation did not affect ($P > .05$) oxygen consumption by PDV, liver, and total splanchnic tissues or hepatic extraction of oxygen.

Discussion

This experiment was designed to determine whether strategies of supplementation with SBM change the timing of nutrient flux in ewes that consume bromegrass hay to simulate supplementation strategies under grazing conditions. Previous reports (Kreikemeier et al., 1993; Goetsch et al., 1994; Whitt et al., 1996) suggest that our sampling protocol was adequate to evaluate relative net flux patterns and differences or similarities among treatments, although a potential limitation to our data is the quantitative assessment of nutrient flux over a 72-h period. This limitation arises from the obvious difficulties associated with bleeding animals at evenly spaced intervals for 72 h. Whitt et al. (1996) conducted three experiments to quantify the patterns of blood flow and net nutrient flux in bovine fed once every 12 h at or near ad libitum intake and sampled every hour for 24 h. Depending on the diet fed and the nutrient evaluated, net PDV and liver fluxes peaked 1.5 to 3 h after feeding and subsequently decreased to average values 5 to 6 h after feeding. Similar work has not been conducted in ruminants fed a wide variety of diets once daily at ad libitum intakes. Goetsch et al. (1994) suggested that low forage digestibility at ad libitum intake should minimize differences in blood flow and nutrient flux with time after feeding.

Forage intake in our experiment was lower than expected, averaging only .79% of BW. The NRC (1985) suggests that 80-kg mature ewes fed at maintenance intake require 2.6 Mcal/d of ME and 122 g/d of CP. In our experiment, ewes fed bromegrass hay alone or with SBM ED or ETD consumed an average of 1.49, 2.19, and 2.07 Mcal/d of ME and 47, 132, or 128 g/d of CP, respectively, over a 3-d period. These data indicate that energy intake was low for all treatments. Even though we did not measure BW change in the present experiment, the low energy and protein in the diet of the control ewes probably resulted in weight loss. This may be supported by the greater arterial concentrations of AAN in the control vs the supplemented ewes if tissue protein was being mobilized.

Ewes fed SBM had a greater intake of bromegrass hay than ewes fed no SBM. This is in agreement with previous experiments (McCollum and Galyean, 1985; Delcurto et al., 1990b; Beaty et al., 1994). Beaty et al. (1994) reported that a SBM-based supplement fed to cattle that were consuming wheat straw resulted in a greater DMI when the supplement was fed daily compared with three times per week. The authors noted that the decrease in intake associated with the three-time supplementation may relate to responses elicited by the large amount of supplement consumed per feeding. In our experiment, feeding 486 g of SBM (DM basis) resulted in a numerically lower forage intake on the day of supplementation compared with daily supplementation. However, forage intake recovered the day after and 2 d after supplementation. In the experiment of Beaty et al. (1994), one effect of feeding three times per week vs daily was a trend for slower rate of indigestible ADF passage in the three-time group. In contrast, Huston et al. (1997) found similar forage intake in pregnant Brangus and Hereford × Brangus cows that consumed winter range in west Texas and were supplemented with the equivalent of .91 kg/d of cottonseed meal fed daily, three times per week, or one time per week. Moreover, these authors noted that cows fed daily varied most in supplement intake presumably because of aggressive competition during a short consumption period. Differences in animal behavior in confinement vs grazing may explain the differences observed between the data of Beaty et al. (1994), Huston et al. (1997), and our experiment.

In cattle, increasing amino acid (**AA**) flow to the small intestine, either by infusing casein into the abomasum (Guerino et al., 1991) or by increasing DMI (Huntington et al., 1988; Glenn et al., 1989), increases net portal release of AAN. Therefore, differences in AAN flux that occurred in our study probably reflect differences in AA flow to the small intestine. Hannah et al. (1991) found that duodenal flow of nonammonia N was greater in steers fed dormant bluestem-range forage and supplemented with dehydrated alfalfa pellets (17.5% CP) or SBM: grain sorghum (27.1% CP) than in steers not receiving supplement. In our experiment, PA difference and net PDV flux of AAN responded with a SBM × sampling day interaction. Within treatment, control ewes and ewes fed SBM ED had similar AAN concentrations across the three sampling days. These observations are consistent with those of Whitt et al. (1996) and Kreikemeier et al. (1993). In ewes fed SBM ETD, PA difference and net PDV flux of AAN were greatest on the 2nd d followed by the 3rd d and were lowest on the 1st d of sampling. The greater flux of AAN on the 2nd d of sampling may indicate that a lag time in SBM digestion and small intestinal protein

Table 4. Hepatic nitrogen uptake and urea nitrogen synthesis

Item	No supplement			SBM, 24 h[a]			SBM, 72 h[b]		
	D1[c]	D2[c]	D3[c]	D1	D2	D3	D1	D2	D3
	mmol/h								
Hepatic α-amino N flux	–26.9	–13.7	–17.6	–51.1	–44.3	–46.4	–41.8	–64.9	–44.9
Hepatic NH_3 N flux	–12.2	–11.8	–12.7	–38.2	–38.7	–39.2	–28.2	–51.9	–31.6
Total[d]	–39.1	–25.5	–30.3	–89.3	–83.0	–85.6	–70.0	–116.8	–76.5
Hepatic urea N flux	23.7	25.5	20.0	52.3	63.5	49.0	39.0	91.5	48.3

[a]SBM was fed once every 24 h.
[b]SBM was fed once every 72 h.
[c]D1, D2, and D3 represent sampling d 1, 2, and 3. All ewes were sampled 3 d in each period so that ewes fed SBM once every 72 h were sampled the day of, the day after, and 2nd d after supplementation.
[d]Total = hepatic α-amino N flux + hepatic NH_3 N flux.

flow occurred, or that AAN release by the PDV peaked some time after sampling stopped on the day of supplementation. If the latter occurred, the concentration of AAN on sampling d 1 may more accurately reflect conditions 2 d after supplementation than on the day of supplementation. Our results indicate that feeding SBM ETD vs ED changes the pattern of AAN absorption across the PDV.

In ruminants that graze dormant or low-quality forage, protein is considered the first-limiting nutrient. Low ammonia N concentrations (<50 mg/L) in the rumen decreased microbial growth (Satter and Slyter, 1974) and reduced the rate of fiber digestion and, consequently, reduced intake. Supplementing protein to cattle and sheep that are consuming low-quality forage has been shown to increase DM digestibility (Guthrie and Wagner, 1988), ruminal fiber digestion (Beaty et al., 1994), and protein flow to the small intestine (Hannah et al., 1991), which were probably the result of improved ruminal N status (McCollum and Galyean, 1985; DelCurto et al., 1990b; Beaty et al., 1994). Moreover, ruminants fed high-protein supplements at infrequent intervals seemed to be able to sustain elevated ruminal ammonia N levels even on days when they were not supplemented (Judkins et al., 1991; Beaty et al., 1994). In our experiment, PA concentration difference and net PDV flux of ammonia N were greater when SBM was fed, but there was no effect ($P > .05$) of frequency of feeding on the overall mean. Pattern of ammonia N was different among treatments. In ewes fed SBM ETD, PA concentration difference of ammonia N was .133, .220, and .156 m*M* on d 1, 2, and 3 of sampling, respectively. These differences are similar to those previously described for net PDV release of AAN. Reasons for these differences warrant further investigation. The greater net portal flux of ammonia N in sheep fed SBM compared with sheep fed no supplement was likely a result of greater N intake and much greater ruminal ammonia N concentration in sheep fed SBM.

Nitrogen uptake by the liver vs hepatic urea N synthesis is shown in Table 4. The sum of AAN uptake plus ammonia N uptake ranged from 25.5 mmol/h for the control ewes on d 2 of sampling to 116.8 mmol/h in ewes fed SBM ETD on d 2 of sampling. Except for the unsupplemented group on sampling d 2, the amount of N taken up by the liver was more than N in urea synthesized. Liver urea N release accounted for 56 to 100% of liver AAN and ammonia N removal. Similarly, Kreikemeier et al. (1993) found urea N release from the liver to be 42 to 63% of the sum of ammonia N plus AAN uptake by the liver. If all the ammonia N taken up is converted to urea N on a net basis, and if a portion of urea N synthesis is from absorbed nucleic acid N (Huntington, 1986), then a portion of the AAN taken up by the liver was converted to some form of N other than urea N. In contrast to our data, studies with beef cattle (Reynolds et al., 1990, 1991; Reynolds and Tyrrell, 1991) indicate that liver urea N release accounted for 110 to 130% of liver AAN and ammonia N removal. Reasons for observed differences between studies are unclear. Results from our experiment and the study of Kreikemeier et al. (1993) suggest that, in sheep, AAN is delivered to extrahepatic tissues in some form other than free AAN. A large fraction of N may be released from the liver as blood proteins (e.g., albumin, fibrinogen, and globulins). Freetly et al. (1993) estimated that in lactating cows .92 mol/d of amino acids are exported as blood proteins. In the present experiment, a range of .25 to .89 mol of N would have been available for hepatic protein synthesis.

Ruminants recycle substantial amounts of N by urea transfer across the rumen wall or via saliva. Bacteria adhered to the rumen wall hydrolyze urea to ammonia and use the ammonia N for bacterial protein synthesis. Ruminants then reabsorb portions of the N in the form of amino acids, nucleic acids, or ammonia. In our experiment, net transfer of urea N to the PDV ranged from 20 to 40% of hepatic production across all treatments. In addition, net transfer of urea N to the PDV was 28 to 52% of intake N in ewes fed bromegrass hay alone, and only 12.6 to 23% when ewes were fed bromegrass hay plus SBM ED. Therefore, as a proportion of intake, urea N return to the

gut was greatest when intake of N was low, which demonstrates the magnitude of importance of N recycling via this route when low N intakes occur. Interestingly, when ewes were fed SBM ETD, net PDV removal of urea N was only 12.3% of N intake on the day of supplementation and averaged 74% on the 2 d following supplementation. Based on the NRC (1985) requirement of 122 g/d of CP (19.5 g/d of N) for ewes fed at maintenance, PDV removal of urea N met 16% of the N requirement in unsupplemented ewes and averaged 31% of the N requirement on d 2 and 3 of sampling in ewes fed SBM ETD. Even though net PDV flux values do not include salivary urea or indicate a specific site of urea transfer, these data suggest that the PDV of ewes fed SBM ETD removed more urea N, which may have sustained elevated ruminal ammonia N concentrations on the 2 d following supplementation. Beaty et al. (1994) suggested that ruminants fed high-protein supplements at infrequent intervals were able to sustain elevated ammonia N levels even on days when they were not supplemented. In addition, because many of the important N transport processes are concentration-dependent (Mazanov and Nolan, 1976), the high arterial urea N concentration on the day following supplementation may have resulted in a greater (more negative) net PDV flux of urea N in ewes supplemented with SBM ETD.

Even though infrequent supplementation seems to increase urea N removal by the PDV on days in-between supplementation, increased urea N output by the liver may represent an energetic cost to the animal. Hepatic ureagenesis has been estimated to contribute 13 to 16% to liver energy expenditure in cattle (Huntington, 1989; Reynolds et al., 1991) and 13 to 19% in sheep (Lobley et al., 1995). These estimates are based on the stoichiometric requirement of four high-energy phosphate bonds per mole of urea synthesized, and they assume six high-energy phosphate bonds per mole of oxygen consumed (Lobley et al., 1995). Lobley et al. (1995) infused NH_4Cl into a mesenteric vein for 5 d at rates of 25 μmol/min or 235 μmol/min. Liver urea N production when the low level of NH_4Cl was infused represented 13% of liver oxygen consumption, and this value increased to 19% when the high ammonia level was infused. In our experiment, liver urea N release was estimated (Lobley et al., 1995) to account for an average of 6.6% of liver oxygen consumption in control ewes and an average of 12.4% in ewes fed SBM. In ewes fed SBM ETD, ureagenesis was estimated to account for 9.4, 17.8, and 11.3% of liver oxygen consumption on d 1, 2, and 3 of sampling, respectively. These data confirm that greater removal of ammonia N by the liver results in increased energetic costs associated with hepatic ureagenesis. Simple linear regression (r = .904) resulted in the following equation.

$$\text{Liver } O_2 \text{ consumption} = 197.5 + (1.84 \times \text{liver urea N release})$$

The high correlation between liver oxygen consumption and ureagenesis may also be indicative of the high energy cost of urea production.

Supplementation of bromegrass hay with SBM did not affect net glucose release by the liver, indicating that glucose was not limited in these ewes. The decrease in net liver lactate removal observed when SBM was fed suggests that increased AAN supply to the liver shuffled the availability of glucose precursors. Similar effects on liver lactate removal have been reported during mesenteric vein infusion of alanine in heifers (Reynolds and Tyrrell, 1991) and during mesenteric vein infusion of propionate in dairy cows (Baird et al., 1980).

Goetsch et al. (1994) fed wethers bromegrass hay with supplemental feedstuffs high in starch, ruminally-degradable fiber, or ruminally-undegradable protein and found no differences in oxygen consumption by splanchnic tissues. In our experiment, feeding SBM increased total splanchnic oxygen consumption by 25%. Conversion of oxygen consumption by total splanchnic tissues to heat production (**HE**) by the factor of 110 kcal HE/mol of oxygen consumed (McLean, 1972) results in an average of 46 kcal/h for the control ewes and 61 kcal/h for ewes fed SBM.

In summary, supplementing SBM to ewes that are consuming bromegrass hay increased intake of hay, CP, and ME. Supplementing SBM ETD numerically decreased hay intake to control levels on the day of supplementation, but hay intake was greater than in controls on d 2 and 3 of supplementation. Release of ammonia N and AAN by the PDV were similar across days in unsupplemented ewes and in ewes fed SBM ED. Ammonia N and AAN release by the PDV were greater on the day following supplementation than on the day of or 2nd d after supplementation in ewes fed SBM ETD. Removal of ammonia N and AAN by the liver mirrored PDV release across all treatments. Urea N release by the liver accounted for 56 to 100% of liver ammonia N and AAN removal and accounted for 5.8 to 17.8% of hepatic oxygen consumption. Oxygen consumption accounted for by hepatic urea production was greatest on d 2 when SBM was fed ETD. Portal-drained viscera removal of urea N was 12% of N intake on the day of supplementation and averaged 74% of N intake 2 d following supplementation in ewes supplemented ETD. These data show the importance of N removal by PDV in ruminants supplemented at infrequent intervals.

Implications

Supplementing soybean meal to mature ewes that were consuming low-quality forage increased forage intake and net portal and hepatic flux of nitrogenous compounds. Even though the pattern of absorption was different when soybean meal was fed every three

days, net flux of nutrients was generally not affected by frequency of protein supplementation. One noted difference was the removal of urea nitrogen by portal-drained viscera; it was greater when soybean meal was fed every three days. This may result in a maintained or improved nitrogen economy for the ruminal microorganisms.

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